

AMENDMENTS TO THE CLAIMS

1. (Original) A method for quantitation of zinc ion in a sample comprising:
 - i) disposing in said sample a donor fluorophore and a carbonic anhydrase protein having conjugated thereto an acceptor fluorophore, wherein the binding of said donor fluorophore to the conjugated carbonic anhydrase is dependent upon the presence of zinc ion complexed to the conjugated carbonic anhydrase;
 - ii) measuring the intensity of a photoluminescence emission of the acceptor fluorophore upon excitation of the donor fluorophore at a first excitation wavelength;
 - iii) measuring the intensity of a photoluminescence emission of the acceptor fluorophore upon excitation of the acceptor fluorophore at a second excitation wavelength;
 - iv) obtaining the ratio of the intensities measured in steps ii) and iii) and;
 - v) relating the quantity of zinc ion in the sample to the ratio obtained in step iv).
2. (Original) The method of claim 1, in which the photoluminescence emissions in step ii) and in step iii) are fluorescent emissions.
3. (Original) The method of claim 2, in which the donor fluorophore is a fluorescent sulfonamide.
4. (Original) The method of claim 2, in which the donor fluorophore is Dapoxyl sulfonamide.

5. (Currently amended) The method of claim 2, in which the acceptor fluorophore is Alexa FluorALEXA FLUOR 594.

6. (Currently amended) The method of claim 3, in which the acceptor fluorophore is Alexa FluorALEXA FLUOR 594.

7. (Currently amended) The method of claim 4, in which the acceptor fluorophore is Alexa FluorALEXA FLUOR 594.

8. (Original) The method of claim 2, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

9. (Original) The method of claim 3, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

10. (Original) The method of claim 4, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

11. (Original) The method of claim 5, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

12. (Original) The method of claim 6, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

13. (Original) The method of claim 7, in which the carbonic anhydrase is human carbonic anhydrase II having a cysteine residue substituted for leucine residue 198 or having a cysteine residue substituted for histidine 36 and the acceptor fluorophore is conjugated to said cysteine residue.

14. (Original) A method for quantitation of zinc ion in a sample comprising:

i) disposing in said sample a donor fluorophore and a fusion protein comprising a carbonic anhydrase protein and a fluorescent protein acceptor fluorophore, wherein the binding of said donor fluorophore to the fusion protein is dependent upon the presence of zinc ion complexed to the conjugated carbonic anhydrase domain of the fusion protein;

ii) measuring the intensity of a photoluminescence emission of the acceptor fluorophore upon excitation of the donor fluorophore at a first excitation wavelength;

iii) measuring the intensity of a photoluminescence emission of the acceptor fluorophore upon excitation of the acceptor fluorophore at a second excitation wavelength;

iv) obtaining the ratio of the intensities measured in steps ii) and iii) and;

v) relating the quantity of zinc ion in the sample to the ratio obtained in step iv).

15. (Original) The method of claim 14, in which the photoluminescence emissions in step ii) and in step iii) are fluorescent emissions.

16. (Original) The method of claim 15, in which the fluorescent protein is Green Fluorescent Protein, Enhanced Green Fluorescent Protein, Yellow Fluorescent Protein, DsRed, or Blue Fluorescent Protein.

17. (Original) The method of claim 15, in which the donor fluorophore is a fluorescent sulfonamide.

18. (Currently amended) The method of claim ~~17~~¹⁶, in which the donor fluorophore is a fluorescent sulfonamide.

19. (Original) The method of claim 16, in which the donor fluorophore is Dapoxyl sulfonamide.

20. (Original) The method of claim 14, in which the sample comprises a living cell.